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Bureau of Laboratories

"Quality Laboratory Science for Healthier People and Communities"

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Effects of Non-Culture Testing Procedures on Public Health

Patricia Somsel, Dr. P.H.
Division of Infectious Diseases
(Letter Reprinted with Permission)

Dear Colleagues:

Advances in technology have led to more rapid detection of pathogens in human clinical samples. Clinical laboratories are increasingly adopting these methods to provide more rapid diagnosis and treatment, with the goal of achieving shorter stays for hospital patients. These are generally positive outcomes for patients and hospitals alike, but decisions to implement non-culture technologies must be made with a full understanding of their limitations, and the broader implications for our communities of their widespread use. The Association of Public Health Laboratories, representing over 700 domestic and international laboratory practitioners has developed a policy statement outlining concerns about the impact of non-culture methods on public health's ability to detect and control disease.

Many of these methods - which include biochemical, DNA-based, immunoassay, and other non-culture platforms - have shown increased sensitivity and specificity over time, and have been touted to eliminate the need for culture. Because culture based- methods are expensive, time consuming, and require laboratory expertise that is increasingly harder to find, laboratory and hospital administrators may be eager to adopt methods that "replace" cultures.

But these non-culture methods have limitations, among them the inability to detect the presence of unsuspected or non-targeted agents. The targeted agents may also undergo genetic changes, which make them undetectable in the non-culture diagnostic, a problem that has been observed several times in the past with rapid flu test kits. Testing for viral respiratory agents by nucleic acid detection or by antigen detection only, for instance, can provide a rapid answer; but careful reading of the package insert of one newly approved multiplexed respiratory viral nucleic acid test reveals that *culture confirmation is still recommended* for negative results. Thus maintaining the ability to perform viral culture is critical. Additionally, the performance of antigen detection flu tests depends upon the level of virus circulating in the community. The positive predictive value is very low prior to the confirmed onset of the flu season.

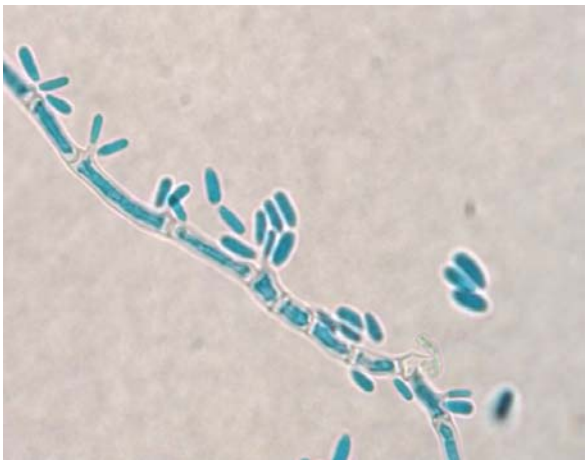
Non-culture based tests will also have a large impact on the ability of the public health system to investigate and control disease and respond to outbreaks in the community. Institution of these rapid methods, if accompanied by elimination of bacterial or viral cultures, may also result in the loss of specimen for follow-up testing; either because the specimen is entirely consumed by the rapid procedure, or it is collected in a medium, which renders it unusable for follow-up testing. This may prove problematic for several reasons. Detection of Shiga toxin by immunoassay methods, without subsequent culture for *E. coli* O157 (or forwarding of positive broths to the public health laboratory) leaves public health without a means to fully characterize and track disease activity. When unexpected results are obtained, for instance, as in a rapid flu test positive in mid-summer, the ability of the public health lab to confirm the performance of the rapid test kit is impossible without a specimen. The full characterization of a true positive in such a setting could be of major significance in the context of novel or pandemic influenza.

Utilization of non-culture based tests if it results in the elimination of culture based testing capability or limited retention of specimen material should be carefully reviewed prior to implementation. The reduction or elimination of isolates flowing into the public health system will have a serious impact on the quality of public health detection, investigation, and control of disease. We appreciate your consideration of these issues when adjusting your test menu.

FUN FUNGI.....
Hormonema Species

Sandy Arduin MT (ASCP) & Bruce Palma MT (ASCP) - Mycobacteriology/Mycology Unit

Last Issues Picture Quiz Answer:



***Hormonema* species**

***Hormonema* Species:**

Hormonema species are often misidentified as *Aureobasidium* species. Although similar in colonial appearance, they can be differentiated microscopically by conidial formation. Colonies are yeast-like, white, tan, or slightly pinkish in color becoming dark brown to olivaceous black with age. Optimum growth temperature for *Hormonema* is 25˚C. Microscopically, hyphae are initially hyaline, septate and thin walled. With age, the hyphae become dematiaceous and thick walled with the cells becoming wider than they are long; often converting into thick walled chlamydospores. Conidia are hyaline, ellipsoidal and vary in size. *Hormonema* conidia are formed asynchronously through percurrent proliferation (successive conidia form through the same opening). Conidia often appear as a cluster around an individual conidiogenous structure, making it difficult to differentiate *Hormonema* from *Aureobasidium* species. *Aureobasidium* species' conidia form synchronously (at the same time) in close tufts with each conidia attaching by a separate denticle to the conidiogenous structure.

Hormonema dematioides is an important wood-bluing fungus associated with the discoloration of coniferous wood or needles. *H. dematioides* has rarely been found to be pathogenic to humans. It has been documented as the cause of subcutaneous phaeohyphomycosis of the hand in an immunocompetent host and the cause of peritonitis in a patient on continuous ambulatory peritoneal dialysis (CAPD). *H. dematioides* has been recovered from pleural fluid, CSF, blood and a surgical wound.

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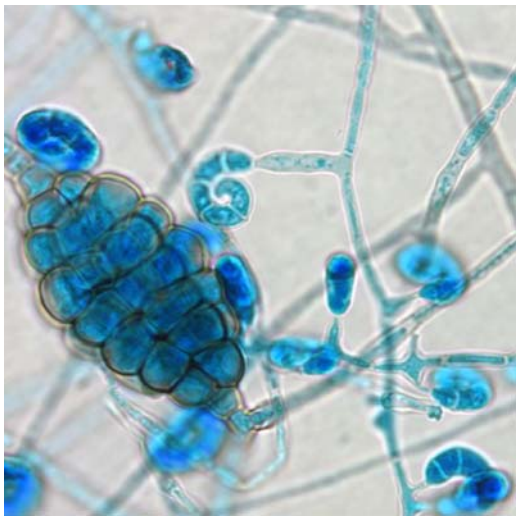
Picture Quiz: What Mould is this?



European Lyme Borreliosis Case Studies

William Crafts, MT (ASCP)
Bacterial & Parasitic Serology Unit

Background:
Lyme disease, now referred to as Lyme Borreliosis (LB), is the most common vector-borne disease in North America and Europe and represents a significant public health concern. *Borrelia burgdorferi* sensu stricto is the only pathogenic *Borrelia* spp. detected in North America whereas *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii* and *Borrelia garinii* are endemic throughout Europe. LB is transmitted by the bite of infected *Ixodes scapularis* (blacklegged or deer tick) and *Ixodes pacificus* (western black legged tick) ticks in the United States and *Ixodes ricinus* ticks in Europe. MDCH reported three cases of European Lyme Borreliosis in Michigan where traditional 2-tier serologic testing, using commercial test kits utilizing *Borrelia burgdorferi* strain B31, was unable to confirm the diagnosis. Most laboratories in the United States (U.S.) are unable to detect antibodies to *B. afzelii* and *B. garinii*, two of the pathogens responsible for European LB. Case 1 describes a resident of Germany visiting the lower peninsula of Michigan, presenting with an erythematous lesion. Case 2 describes a resident of Michigan who returned from touring and hiking in Switzerland and presented with fatigue and chest pain. Case 3 is a resident of Germany visiting the lower peninsula of Michigan and presenting with abdominal pain, vomiting and fatigue. These cases support the importance of obtaining complete travel history, especially to destinations outside the U.S., as well as clinical signs and symptoms to aid in the selection of appropriate LB assays. This will improve the diagnostic capabilities of the laboratory and provide sensitive and specific test results to clinicians.

Case 1
In July 2007, a four-year old boy presented to a Michigan clinic with low-grade fever (99.1ºF) and a large (12 x 5 cm) erythema migrans target lesion located on the right lower chest. The patient resides in Kroenberg, Germany and experienced repeated tick bites. Serologic testing at MDCH for LB utilizing *B. burgdorferi* B31 strain, based on EIA, revealed a reactive polyvalent whole-cell enzyme-linked immunoassay (EIA index value 3.41) and negative IgM and IgG immunoblot. Repeat EIA and immunoblot testing at Trinity Biotech/MarDx Diagnostics, Carlsbad, CA (MarDx) utilizing an antigen mixture of *B. afzelii* (PKO strain), the OspC antigen of *B. garinii* (G2 strain) and *B. burgdorferi* (VisE antigen) was EIA IgM and IgG equivocal. Using the German "MIQ" interpretive criteria, the IgM immunoblot was positive and IgG immunoblot indeterminate confirming early onset LB most likely due to *B. afzelii*. The patient was treated with amoxicillin and fully recovered.

Case 2
In December 2007, a 57 year-old woman presented with abdominal rash, fatigue, left-sided chest pain and difficulty breathing upon returning to Michigan from a hiking trip in Switzerland. The rash began as a two-inch erythematous lesion with clear margins that eventually spread to the entire abdomen and lower back. The rash was originally treated with antibacterial, antifungal and cortisone cream with no improvement. A skin biopsy showed perivascular and interstitial inflammation containing lymphocytes, occasional neutrophils and eosinophils. Serologic testing for LB at MDCH revealed a reactive polyvalent whole-cell enzyme-linked immunoassay (EIA index value 1.45) and negative IgM and IgG immunoblot. The patient was prescribed amoxicillin and clavulanic acid but on day six, returned to her physician reporting myalgia, fatigue and new lesions on the face, thigh and buttocks. Repeat EIA and immunoblot testing at MarDx utilizing European strains antigens was EIA IgM positive and IgG positive. Using the German "MIQ" interpretive criteria, the IgM and IgG immunoblots were both positive confirming the diagnosis of LB most likely due to *B. afzelii*. The patient was subsequently treated with doxycycline and fully recovered.

Case 3

In July 2008, a seven-year old girl and sibling of Case 1 presented to a Michigan clinic with abdominal pain, vomiting, and fatigue. The patient resides in Kroenberg, Germany and experienced repeated tick bites. Serologic testing for LB at MDCH revealed a negative polyvalent whole-cell enzyme-linked immunoassay (EIA index value 0.64), a weakly reactive IgM and negative IgG immunoblot. Repeat EIA and immunoblot testing at MarDx was EIA IgM positive and IgG negative. Using the German "MIQ" interpretive criteria, the IgM immunoblot was positive and IgG immunoblot negative confirming the diagnosis of early onset LB. Due to the strong reaction to *B. garinii* OspC antigen, the infection was most likely due to *B. garinii*. The patient was treated with amoxicillin and fully recovered.

Discussion:

Clinical signs and symptoms, date of onset, and travel history originally provided to MDCH was inaccurate and incomplete in all three cases described above. Further investigation revealed all patients traveled or resided in Europe and were potentially exposed to ticks. A third generation EIA, designed for use in Europe utilizing the native purified antigens of *B. burgdorferi* (B-31 strain and VisE protein), *B. afzelii* (PKO strain) and *B. garinii* (G2 strain), was IgM and IgG equivocal in Case 1, IgM and IgG positive in Case 2, and IgM positive and IgG negative in Case 3. Results were interpreted according to the manufacturer's instructions, lyme index value ≤ 0.49 (negative), 0.5 – .99 (equivocal), and ≥ 1.0 (positive). A second generation western immunoblot (WB), which incorporates a combination of partially purified antigens of *B. afzelii* (PKO), purified OspC (outer surface protein) of *B. garinii* G2 strain and *B. burgdorferi* VisE antigen, was IgM positive and IgG indeterminate Case 1, IgM and IgG positive in Case 2, and IgM positive and IgG negative in Case 3. The strong IgM reaction to OspC in Case 3 indicates infection with *B. garinii* (Table 2). Current CDC immunoblot interpretive guidelines (Engstrom et. al. for IgM and Dressler et. al. for IgG) were established using *B. burgdorferi* sensu stricto strain 297 and strain G39/40 respectively on patient sera obtained exclusively from North American LB patients (1, 2). OspC and other dominant protein expression vary amongst *Borrelia* species and strains, hence, CDC immunoblot interpretive guidelines are not applicable for the diagnosis of European LB. Immunoblot interpretive criteria have not been fully standardized in Europe and reactions to specific proteins are dependent upon the *Borrelia* genospecies utilized in each assay. Trinity Biotech immunoblot assays use the German interpretive criteria (MiQ 12/2000) and the criteria in place for the rest of Europe (modified MiQ12/2000) for determination of positive and negative reactions (3, 4). In early LB when the immunologic response is weak, the presence of two or more of the following IgM bands is considered positive; p17, p39, p41, *B. afzelii* (PKO) p22 OspC or *B. garinii* 22 kD OspC. For IgG, the presence of any three or more of the following bands is considered positive, p14, p17, 22kD, OspC, p30, p39, p43, p58, p100, *B. garinii* OspC, *B. burgdorferi* VisE. These criteria are more applicable to the immunologic response observed in European LB patients.

Conclusion:

The diagnosis of LB is based on clinical signs and symptoms, travel history to endemic locations, possible tick exposure and laboratory testing. Variations in *B. burgdorferi* sensu stricto strains or recombinant antigens utilized in commercial and in-house EIA and WB assays, poor test sensitivity and specificity, lack of standardized immunoblot interpretive guidelines and lack of accurate clinical information provided to the testing laboratory, contribute to the difficulty surrounding the serologic diagnosis of LB. The use of recombinant antigens from several different *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* strains targeting specific proteins appear to enhance specificity of serologic assays (5). Screening assays utilizing whole cell sonicated *B. burgdorferi* sensu stricto antigens are sufficient to capture most cases of LB. To enhance antibody detection, immunoblot assays should incorporate recombinant proteins from all three *B. burgdorferi* sensu lato genospecies. A collaborative effort between clinicians and laboratory personnel is imperative

to ensure appropriate testing is performed and proper interpretive guidelines are applied. These cases support the importance of obtaining a complete travel history, especially to destinations outside the U.S., as well as clinical signs and symptoms to aid in the selection of appropriate LB assays. This will improve the diagnostic capabilities of the laboratory and provide sensitive and specific test results to clinicians. Serologic studies using defined serum from LB patients worldwide should focus on the optimization and standardization of *Borrelia* antigens utilized in screening and confirmatory assays.

TABLE 1
Lyme EIA (Wampole) and IgM/IgG Immunoblot (MarDx) Results Using *B. burgdorferi* B31 strain (MDCH)

Patient	Polyvalent IgM/IgG Lyme EIA Index Value (LIV)*	EIA Interpretation	IgG Immunoblot Interpretation and Bands Observed†	IgM Immunoblot Interpretation and Bands Observed†
CASE 1	3.4	POS	NEG (p41)	NEG (p41)
CASE 2	1.4	POS	NEG (p41)	NEG (p18, p41)
CASE 3	0.6	NEG	POS (p23, p41)	NEG (p41)

*****Cutoff values: ≤ 0.90 = negative (NEG); 0.91 – 1.09 = equivocal (EQ); ≥ 1.1 = positive (POS).
LIV = Lyme index value
†Detects IgM or IgG antibodies to *B. burgdorferi* B31 strain antigens.

TABLE 2
European Lyme EIA & Immunoblot Results (MarDx)

Patient	IgM Lyme EIA Index Value (LIV)*	IgM EIA Interpretation	IgG Lyme EIA Index Value (LIV)*	IgG EIA Interpretation	IgM Immunoblot Results and Bands Observed†	IgG Immunoblot Results and Bands Observed‡
Case 1	0.9	EQ	0.8	EQ	POS* (p41,OspC)	IND (p14,p22,p41)
Case 2	1.1	POS	2.5	POS	POS* (p22, OspC)	POS (p14,p17, p30,p41,p43, OspC)
Case 3	1.4	POS	0.4	NEG	POS* (p22,p41,OspC)	NEG* (p41)

* Cutoff values: ≤ 0.49 = negative (NEG); 0.5 – 0.99 = equivocal (EQ); ≥ 1.0 = positive (POS).
IND. = Indeterminate
†Detect IgM antibodies *to B. afzelii*, (PKO), *B. garinii* OspC antigens.
‡Detect IgG antibodies to *B. afzelii*, (PKO), *B. garinii* and *B. burgdorferi* VisE antigens.

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Michigan Newborn Screening Annual Report

The following are excerpts from the Executive Summary of the 2007 Annual Report of the Michigan Newborn Screening Program.

The Newborn Screening (NBS) annual report provides an overview of the Michigan Program, target outcomes, screening performance metrics, and quality assurance information. This report differs from the one released in 2006 in several ways. First, this is an abridged report in that it does not include appendices which have not changed since 2006 including the NBS research guidelines, supportive legislation, or website description.[1] Second, this report includes a chapter providing in-depth information on a single NBS condition, congenital hypothyroidism (Chapter IV). In this chapter an overview of CH screening, past and present, is provided, information on definition, diagnosis, and treatment are provided, and updates on ongoing CH related NBS program evaluation research are provided. Subsequent NBS annual reports will each include a chapter detailing a different disorder each year. Third, regional prevalence estimates are not provided (numerically or graphically) because these estimates will not have changed significantly from 2006 to 2007.

Since the program began in 1965 with the screening for phenylketonuria, 48 additional disorders have been added to the screening panel, millions of infants have been screened, and approximately 4,000 have been diagnosed with disease included in the NBS panel.

In 2007, of 123,477 infants screened, 123,181 were Michigan residents; 0.16% (200 of 123,181 resident newborns screened) of them were diagnosed as having a disease. More than half of the 127 cases having treatment information reported were treated within two weeks of life.

Developments occurring in 2007:

- The NBS program implemented a NICU/Low Birth Weight screening protocol for infants weighing less than 1800g at birth.
    - In 2007, the detection of congenital hypothyroidism increased nearly three fold among infants screened via the NICU/Low Birth Weight protocol.
    - The NBS program is currently considering altering inclusion criteria to expand eligibility for this protocol.
- As of October 1st, 2007, the fee for the NBS card was increased to $85.61 as recommended by the Quality Assurance Advisory Committee.
- Early hearing detection was officially added to the NBS panel in 2007.
- Screening for Cystic Fibrosis began October 1st, 2007.
    - Seven cases were detected out of 132 positive screens in 2007.
- A courier service was implemented in 2007 to transport dried blood spot samples from hospitals to the NBS laboratory in order to reduce time to diagnosis and accordingly time to treatment for conditions included in the NBS panel.
    - At the end of 2007 38% of the birthing hospitals sent 58% of the NBS samples by courier;
    - As of July, 2008, 90% of hospitals are sending 93% of the specimens via courier. Thus, we expect significant improvements in time from birth to lab receipt of specimens in 2008.
- The NBS Follow-up Program began matching live birth records provided by the Division of Vital Records and Health

[1] The 2006 and 2007 NBS Annual Reports are available at www.michigan.gov/newbornscreening

Statistics to NBS records in order to identify potentially unscreened infants.
    - Thus far, more than 99% of live birth records have been matched to NBS records.
- The NBS Follow-up Program implemented a Three Year Follow-up Protocol to confirm the diagnosis of permanent congenital hypothyroidism among borderline cases (those having pre-treatment serum thyroid stimulating hormone levels in the bottom 15th percentile) after age three years.
    - Thus far, half of the cases followed up are thought not to have permanent congenital hypothyroidism, although tracking is ongoing.
- A pilot second tier congenital adrenal hyperplasia (CAH) screen added to the NBS program in August 2006, continued through 2007. The second tier screen is being run in tandem with the traditional method.
    - Compared to the traditional method, addition of the 2nd tier test could result in a ~95% reduction in false positive screening results for CAH.
    - Among non-NICU births the positive predictive value (number of true cases of disease out of the total number of positive screens) increased by more than 7 fold and the decrease in the false positive rate (number of false screens out of the total number of screens) was equally impressive for the second tier screen.
    - A modified tier-based screening algorithm is currently under consideration.
    - However, medical decisions are not based on these results at this point; follow-up of newborns continues to be based on results of the initial screening method.

Newborn Screening Program Hosts Newborn Screening Family Recognition Day

Teresa Miller and
Kevin Cavanagh, Ph.D.
Division of Chemistry and Toxicology

The MDCH Newborn Screening Program has recently launched the Parent and Family Network Initiative. A kick-off event for the initiative, NBS Family Recognition Day, was held on Saturday, September 6, 2008, at the Impression 5 Science Center in Lansing. This event recognized children, who had disease detected through NBS, and their families. The event featured hands-on activities and workshops for children of all ages, as well as educational exhibits for parents.

The Newborn Screening Program assures that all newborns in Michigan are screened for forty-nine treatable, disorders. All infants with positive tests receive confirmatory diagnosis and treatment. This network initiative was developed to ensure continuing education of parents and families of diagnosed children. It also encourages active participation in the policies and programs affecting the care of their children.

Carrie Langbo and Violanda Grigorescu, M.D., from the Bureau of Epidemiology, hosted this event with Frances Pouch Downes, Dr. P.H., Laboratory Director, Harry Hawkins, manager of newborn screening laboratory, Teresa Miller, and Swarup Khiroya. Other MDCH laboratory staff provided assistance for the event.

The newborn screening laboratory provided a hands-on activity allowing attendees the opportunity to extract their own DNA. The DNA was placed in a necklace amulet as a souvenir of the event. This expanded the families' perspective of the newborn screening program and gained support for the initiative. Other activities included a microscopic view of normal red cells and those from a patient with

sickle cell anemia, a tabletop display on how to collect and prepare dried blood spots for the NBS lab, and a history of the Newborn Screening program. Tabletop displays from several other important MDCH initiatives were also provided to attendees.

Over 100 family members, of children identified through newborn screening, participated in this event along with ten vendors. Everyone in attendance experienced an appreciation for the Newborn Screening program provided by MDCH and the State Of Michigan.

Bureau of Laboratories Vision

The Bureau of Laboratories is a stronger, more diverse team within an integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

Bureau of Laboratories Mission

We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

Director, Bureau of Laboratories
Frances Pouch Downes, Dr.P.H.

Editor
Susan L. Shiflett